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Potential of Watermelon (*Citrullis Lanatus*) Peel Extract in Attenuating Benzo[*a*]Pyrene Exposure-Induced Molecular Damage in Liver Cells *in vitro*

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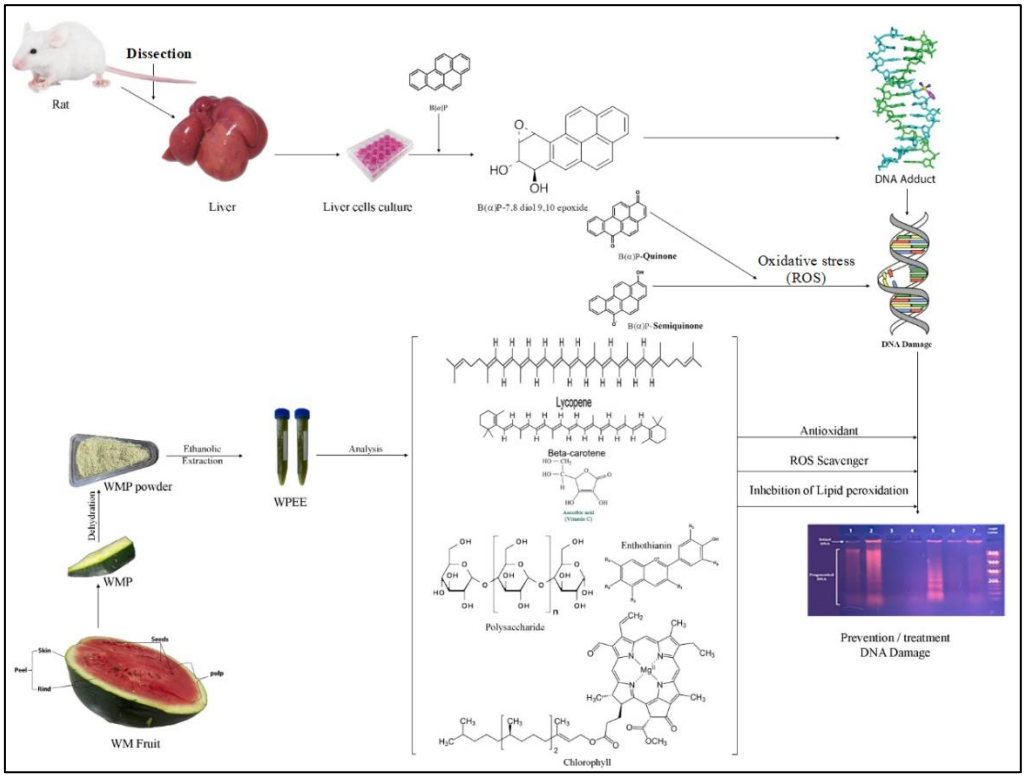
Abstract

Benzo[*a*]pyrene (B[*a*]P), a ubiquitous and prototypical environmental and dietary toxicant, promotes oxidative stress and DNA damage. Plant parts such as watermelon (*Citrullis Lanatus*) peel have potent antioxidative activity that protects cells from oxidative stress and cellular damage. In the present study, we evaluate the bioactive compounds content and antioxidant activity of watermelon peel ethanolic extract (WPEE) and its potential role in attenuating B[*a*]P exposure-induced molecular damage in liver cells *in vitro*. WPEE showed high amounts of bioactive compounds, which include polyphenols, polysaccharides, lycopene carotenoids, flavonoids, tannins, anthocyanins, chlorophyll and some vitamins such as A, C and E besides high antioxidant activity. Liver cells cultures subjected to 10 µM of B[*a*]P leads to many adverse effects including cytotoxic effect (inhibition the cell growth) and damage to macromolecules, such as DNA and RNA measured by Electrophoretic pattern. This induction was markedly reduced after WPEE Co-treatment by the concentrations 25, 50, 75 and 100 µg.ml^{-1} which exhibited a dose-dependent efficacy. In conclusion, B[*a*]P-induced cytotoxicity occurs through DNA damage, cell cycle arrest and ROS production. WPEE could reverse some of these B[*a*]P-mediated alterations and therefore be effective natural compounds against the adverse effects of B[*a*]P in liver cells.



Keywords: Watermelon peel; Bioactive compounds; Antioxidant activity: Liver cells culture; Apoptosis; RNA.

1. Introduction

Benzo[*a*]pyrene (B[*a*]P) is part of a class of chemicals called polycyclic aromatic hydrocarbons (PAH). PAHs usually occur as complex mixtures, not as single compounds. B[*a*]P is listed as a hazardous substance under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), has been found at 524 hazardous waste sites on the National Priorities List (NPL), and is ranked number 8 out of 275 chemicals on the Priority List of Hazardous Substances for CERCLA [1]. It is ubiquitously pollutant present in air, water and terrestrials environments [2]**.** Wherefore, peoples could be exposed to B[*a*]P through many eating, breathing, contacting and drinking sources. Sources of B[*a*]P in the diet are barbecued/grilled/broiled and smoke cured meats, roasted and baked foods, fried foods and vegetables grown in contaminated soil [3, 4]. B[*a*]P is also present soil and its levels in this case vary depend on the proximity to roads, combustion sources, use of sewage or sludge derived amendments on agricultural lands etc. Use of contaminated water sources with petroleum spills, road run off, industrial wastewater also increases the risk of exposure to B[*a*]P [5] . Sources of B[*a*]P in ambient air are industrial and residential emissions such coal-tar and asphalt production plants, smokehouses, local trash and farm plants burning, motor vehicle exhausting, cooking and commercial heating by organic fuel, tobacco smoking [6, 7]**.** Exposure can also occur via the use of dermally applied pharmaceutical products such as coal tar-based shampoos and treatments for eczema and psoriasis [4, 8]. Use of contaminated water by petroleum spills, road run off and industrial wastewater also increases the risk of exposure to B[*a*]P [9]**.** B[*a*]P have been shown to be toxic, mutagenic and/or carcinogenic by extensive *in vivo* [10-12] and *in vitro* studies. Also, B[*a*]P exposure is associated with the development of liver toxicity and carcinogenicity in all vertebrata [2, 10, 11, 13, 14]. It is known that B[*a*] P is considered a promutagen i.e. its mutagenicity dependent upon metabolic activation. B[*a*]P is metabolized by both phase-I and phase-II enzymes to form arene oxides, phenols, quinones, dihydrodiols, and epoxides, and their polar conjugates with glutathione, sulphate and glucuronide [15]**.** B[*a*]P-diol epoxide metabolites interact preferentially with the exocyclic amino groups of deoxyguanine and deoxyadenine in DNA to form adducts [2, 15-17]. Adducts may give rise to mutations unless these adducts are removed by DNA repair processes prior to replication. Transversion mutations (e.g., GC→TA or AT→TA) are the most common type of mutation found in mammalian cells following diol epoxide exposure [18]**.** The Fixation of a biochemical changes by cell proliferation is considered the next step correlated with B[*a*]P mutagenicity [2]**.** Several years ago, many studies were conducted that dealt with the therapeutic effects of some synthetic chemical compounds on the toxic and carcinogenic effects of B[*a*]P [19, 20].

However, many scientific and economic considerations represented in insufficient efficacy, undesirable side effects, and high costs have limited the use of such compounds. Thus, there is a need to explore alternative therapies particularly from natural sources as these are cost effective and possess minimal side effects. Our previously studies with the others have been used sucessively some plant parts including mulberry leaves, onion skin, prickly pear peel, turmeric rhizomes and eggplant peel [21-24] in protecting the liver disorders and toxicity induced by B[*a*]P. In the current study, the research will be extended to include other plant parts that may be more effective in protecting the liver damage caused by exposure to carcinogenic compounds.

Watermelon (*Citrullus lanatus*) is the main world crop in the *Cucurbitaceae* family followed by cucumber, melon and pumpkin and is grown all over the world. Egypt was among the largest watermelon producing countries in the world, with a production of 1.78 million tons [25]. Most of the people are eat watermelon because it provides the body with the needed vitamins, minerals and fiber. However, only the fleshy pulps of watermelon fruits are consumed leaving behind the seeds and the peels. Watermelon peel (see Fig 1.)is generated from restaurants, small scale fruit juice producers, fruit sellers, food beverages processing lines and these wastes are not much being reused. This result to great amount of wastes (peels) generated and discarded. Getting rid of these peels can have serious environmental impact that has been very difficult to solve.

Several studies indicated that Watermelon peel contain many nutrients such proteins, dietary fiber and carbohydrates; minerals such iron, manganese, zinc, potassium, calcium, magnesium and phosphorous and vitamins such A, B and C [26, 27]**.** Also, many bioactive compounds such phenolics, chlorophyll, flavonoids, tannin, saponins, steroids, triterpenoids and alkaloids have been found in mulberry leaves [28, 29]**.** Such bioactive compounds found in watermelon peel possesses medical benefits, including antioxidant properties, antibacterial, hypoglycemic, antiviral, hypotensive properties, anticarcinogenic, neuroprotective functions, enhance the immune system function, reduced risk of heart disease, improved blood pressure, decrease LDL oxidation, and exert a cardio protective effect [30-34]**.** Based on all such previous information, the current study was designed to explore the potential effects of watermelon (*Citrullis Lanatus*) peel extract in attenuating Benzo[*a*]pyrene exposure-induced biochemical and molecular damage in liver cells *in vitro*.

2. Materials and Methods

2.1. Materials

2.1.1. Watermelon Fruits

Watermelon fruits were bought from the local markets, Shebin El-Kom City, Minoufiya University, Egypt.

2.1.2. Chemicals

Benzo[*a*]pyrene (B[*a*]P0, Dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent and bioactive compounds standard [gallic acid (GA), catechine (CA), α-tocopherol and Butylated hydroxytoluene (BHT)] were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cell culture instruments and media were purchased from the

companies shown next to each of them in the research methods. All other chemicals (Except as otherwise stated), reagents and solvents were of analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

2.1.3. Equipment's

Absorbance (Abs) and fluorescence (FL) for different assays were measured using Labo-med. Inc., spectrophotometer, CA and Schematzu fluorescence apparatus, Japan, respectively. High performance liquid chromatography (HPLC) system (Thermo Separation products, San Jose, CA) was used in the present study with the following units: Consta Metvic 4100 pump, UV/VIS Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. Columns (Alltech, Deerfield, IL) were used for separations as follow: a reversed-phase water Adsorbosil C18 (5 µM, 100 mm × 4.6 mm I.d.) for vitamin C and normal Ultrasphere Si (5 µM, 250 mm × 4.6 mm I.d.) for analysis of vitamins A and E.

2.2. Methods

2.2.1. Preparation of Watermelon Powder

Watermelon fruits were thoroughly washed to remove dust and sand particles after which it was sliced using a home choice knife. The pulp was carefully scraped off to obtain the peel (skin and rind) which was chopped into pieces with a chipping machine. The peel chips were dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., PA) at two stages 50°C for 5 h followed by 40°C for 10 h until arriving by the moisture in the final product to about 10%. The material that passed through an 80 mesh sieve was retained, kept in polyethylene bags, stored in refrigerator at 40C and used for different experiments later.

2.2.2. Preparation of Watermelon Peel Extract

Watermelon powder was used for its ethyl extract preparation such as mentioned in our previous study, [35] as follow: A 20 g from Watermelon powder plus 180 ml methanol (80%, v/v) were homogenized and transferred to a beaker and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of was removed under reduced pressure at 45°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany.

2.2.3. Chemical Analysis of Watermelon Powder

Watermelon peel samples were analyzed for proximate chemical composition including moisture, protein (T.N. × 6.25, micro - kjeldahl method using semiautomatic apparatus, Velp company, Italy) , fat (soxhelt miautomatic apparatus Velp company, Italy , petroleum ether solvent), ash, fiber and dietary fiber contents were determined using the methods described in the AOAC [36]**.** Carbohydrates calculated by differences: Carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber). Total energy (Kcal/100 g) of watermelon peel samples was calculated according to Insel, *et al.* [37] using the following equation: Total energy value (Kcal/100 g) = 4 (Protein % +carbohydrates %) + 9 (Fat %).

2.2.4. Bioactive Compounds Determination

Total phenolics in watermelon ethanol extract (WPEE) were determined using Folin-Ciocalteu reagent according to Singleton and Rossi [38]. Results are expressed as gallic acid equivalents (GAE/g of dry extract). The total carotenoids in in WME extract were determined by using the method reported by Litchenthaler [39] and results are expressed as β-carotene equivalents /g of dry extract).Total flavonoids contents in WME were estimated using colorimetric assay described by Zhishen, *et al.* [40]. Total flavonoid contents were expressed as catechin equivalent, mg CAE/g of dry extract. Total anthocyanins in WME were determined such as described by Sharif, *et al.* [41]. The total content of anthocyanins in the sample was expressed as mg Cyanidin 3-glucoside, CCy3G equivalent/ g of dry extract. Total polysaccharides were determined in WME according to the method of Harshal and Priscilla [42]**.** Starch was used as a standard and the results were expressed as mg/g of dry extract. Lycopene was determined such as described by Suwanaruang [43] and was expressed as µg/g of dry extract. Tannins were determined in GAE by the method of Van-Burden and Robinson [44] and expressed as mg catechine per g of dry extract. Total chlorophyll content was determined I WME using portable chlorophyll meter hand-hold plant nutrient meter hand-held chlorophyll analyzer, Model, Chlorophyll meter SPAD-502 Plus, Sasha, China and data were expressed as µg/ g of dry extract.

2.2.5. Antioxidant Vitamins Determination

Vitamins (A, C, and E) were extracted from the WPEE according to the methods described by Epler, *et al.* [45]**,** Moeslinger, *et al.* [46] and Hung, *et al.* [47] and analyzed by HPLC techniques, respectively. Under the chromatographic conditions used, mean values ±SD of vitamins A, C and E recoveries were 89.56± 2.56, 90.65 ±2.54 and 86.56±1.98%, respectively.

2.2.6. Antioxidant Activity Determination

Antioxidant activity of WME extract and standards (α-tocopherol) were determined according to the *β*-carotene bleaching method following a modification of the procedure described by Marco [48].

2.2.7. Molecular Biology Experiments

2.2.7.1. Ethical Approval

Biological model, rats, used in experimental design of the study was ethically approved by the Scientific Research Ethics Committee (Animal Care and Use), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval no. 07- SREC- 01-2021).

2.2.7.2. Animals

Animals used in this study, adult male albino rats (170±7.5 g per each) were obtained from Experimental Animal Unit (EAU), Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt. Rats were housed individually in wire cages in a room maintained at 25 ± 5 ^{0}C, relative humidity (51±5%), a 12-hr lighting cycle and kept under normal healthy conditions. All rats were fed on basal standard diet formulated such as for one-week before starting the experiment for acclimatization.

2.2.7.3. Preparation of Rat Liver Cells Culture

Rat liver cells culture were prepared according to the method mentioned by Elhassaneen [2]. with some few modifications by Fila, *et al.* [29] . Briefly, rats were anesthetized in diethyl ether (Sigma Chemical Co., St. Louis, MO) then dissected in a sterile medium inside the UV unit and separated the liver in a petri dish (Baxter Healthcare Corp., McGaw Park, IL) containing Hank's Balanced Salt Solution (HBSS; Sigma Chemical Co.), Livers were excised to a 60 ×15 mm and other tissues unless livers were cut away and the HBSS were removed. The livers were minced with a sterilize scissors and resuspended in RPMI-1640 (Sigma Chemical Co.) adjusted to 330 mOs/kg and supplemented with 25 mM 2-[4-(2-hydroxyethyl) piperazin-1-yl] ethane sulfonic acid buffer (HEPES), 2 mM L(+)glutamine, 100 IU/ml penicillin, 100 mg/ml streptomycin and 10% fetal Calf serum (FCS; all from Sigma Chemical Co.) to give a concentration (10^5 cells/ml).

2.2.7.4. Experimental Design

Liver cultures of rats were seeded at 100 µl (10^3 cells/well) of 96 flat tissue culture plates. A 100 µl of RPMI-1640/FCS growth medium was added to each well. Seeded wells divided into to the following groups: group 1: seeded wells without any addition (as a control negative replicates), group 2: seeded wells with 100 µl of DEMSO (as solvent replicates), group 3: seeded wells with 100 µl (10 µM) of B[*a*])P (as a control positive replicates), groups 4, 5, 6 and 7: seeded wells with 100 µl of B[a]P and 100 µl of WPEE by the concentrations 25, 50, 75 and 100 µg/ml (as treated replicates), respectively. All plates were incubated at 27°C for 24 h in the presence of 5% CO_2 tension. The plates were prepared for molecular assays.

2.2.7.5. Molecular Analysis

2.2.7.5.1. Electrophoretic Pattern of Nucleic Acids (DNA and RNA) Electrophoresis of Lysate Cells

Electrophoresis of lysate cells used to evaluate RNA pattern according to the method described by Hassab, *et al.* [49]**.** A piece of 10 mg of liver, from mice was excised immediately after perfusion. Yellow tips were used to gently crush the hepatic tissues in eppendorf tubes before lysing them in 200l lysing buffer (50mMNaCL, 1mMNa2 EDTA and 0.5 percent SDS, pH 8.3). Gel Preparation: Gel was prepared using 1.8% electrophoretic grade agarose. The agarose was boiled with tris borate EDTA (TBE) buffer (1xTBE buffer; 89 mMTris, 89mM boric acid, 2mM EDTA, pH 8.3). The agarose mixture was then treated with 0.5 microgram/ml ethidium bromide at 40°C. Before loading samples, the gel was poured and allowed to harden at room temperature for 1 hour. 20µl of tissue lysate were put into gel wells, along with 5µl of loading buffer, to identify the electrophoretic pattern of nucleic acids in tissue lysate. Electrophoresis was performed on (Biomtra Standard Power Pack P25) for 2h. at 50V in gel buffer where DNA and RNA were visualized using a 312 nm UV trasilluminator (Cole-Parmer; Cole Parmer instrument Co. Chicago, USA). The intensity of RNA was measured by gel pro analyzer program as maximal optical density.

2.2.7.5.2. DNA Extraction and Apoptosis

Nucleic acids extraction based on salting out extraction method, and modification introduced by Hassab and EL-Nabi [50]**,** where a piece of 10 mg of tissue was squeezed and lysed in 600 µl lysing buffer and was shake gently. The mixture was kept overnight at 37ºC. For protein precipitation, an amount of 200 µl of saturated Nacl was added to the samples. After that, they were gently shaken and centrifuged for 10 minutes at 12000 rpm. A new eppendorf tube was used to transfer the supernatant. The DNA in the supernatant was precipitated by 700 µl cold isopropanol. The mixture was inverted numerous times until fine nucleic acid fibres emerged, then centrifuged at 12000 rpm for 10 minutes. The pellets were rinsed with 500 l of 70% ethyl alcohol and centrifuged at 12000 rpm for 5 minutes after the supernatant was collected. The alcohol was decanted or tipped out after centrifugation, and the tube was blotted with Whatman paper or cleans tissue until the pellets seemed to be dry. For 30 minutes, the pellets were re-suspended in 50 l of TE buffer (10 mMtris, 1 mM EDTA; pH 8) supplemented with 5% glycerol and 15 l of loading

mixture (5µl of RNAse and10 µl loading buffer). Finally, the samples were loaded into gel-wells for detection of apoptosis.

2.2.7.6. Statistical Analysis

All tests/measurements were done in triplicates and presented as mean± SD (standard deviations). Statistical analysis was performed using Student *t*-test and MINITAB 12 computer program (Minitab Inc., State College, PA).

3. Results and Discussion

3.1. Proximate Composition of Watermelon Peel Powder

Data in Table (1) shows the proximate chemical composition and total energy value of watermelon peel powder. From such data it could be noticed that carbohydrates were the largest compound (76.89 %) followed by total protein (7.12 %), ash (3.04 %), crude fiber (2.77 %) and crude fat (0.32 %). Such data are in accordance with Fila, *et al.* [29] who found that the watermelon rind contains an appreciable and varied amount of protein ranged 5.01 to 7.11g/100 g dw which is due to the difference in species. As reported by Pons [51] the appreciable amount of protein in watermelon rind might be the reason for its high citrulline content, an amino acid that plays an important role in human body's urea cycle. Also, Hoque and Iqbal [52] used watermelon rind for the preparation of cake due to its high content of all nutrients, especially protein, ash and carbohydrates. They found that good quality watermelon rind powder cake may be processed incorporating 10.0% watermelon rind powder into the formulation of plain cake for improved nutritional value and other aspects i.e. physic-chemical properties and organoleptic evaluation. Furthermore, which agrees with the results obtained in the study that the rind of watermelon contained higher levels of protein than the pulp these peels in combination with other products can be used to fortify protein content of locally formulated diet for children [25] On the other side, although watermelon peel is represent low-calorie foods however its carbohydrate content is generally high. Such observation are agree with earlier work by Shiela [53] who reported that fruits including watermelon are not very good sources of fats and are thus recommended as part of weight reducing diets as observed in the peel. From all the above studies and others it could be concluded that the chemical composition of watermelon peel vary with one or combination of the following factors, species, season, geographical location and applicable agricultural services [29]. Additionally, the nutrients in the Watermelon peel which is the part always discarded, can contribute immensely to recommended daily allowance and maintenance of good nutritional status and hence good health for human.

3.2. Bioactive Compounds and Vitamins Content, and Antioxidant Activity in Watermelon Peel Ethanol Extract (WPEE)

Bioactive compounds and vitamins content and antioxidant activity of WME were shown in Table (2). From such data it could be noticed that phenolics were the most largest bioactive compound (4.01 mg GAE/g dry extract) followed by Carotenoids (1.26 mg BCE/g dry extract), Flavonoids (1.12 mg CE/ g dry extract), Tannins (0.92 mg CE/g dry extract), Polysaccharides (0.86 mg SE/ g dry extract), Anthocyanin's (0.52 mg, CCy3G E/100g dry extract), Chlorophyll (85.20 µg/g dry extract) and lycopene (80.51 µg/ g dry extract). Also, the content of antioxidant vitamins were recorded 2.76, 0.89 and 0.37 mg/g dry extract of vitamins A, C and E, respectively. Furthermore, WPEE exhibited high level of antioxidant activity (AA, 74.50 to 83.61 % of a-tocopherol (50mg/ml) and BHT (50mg/ml), respectively. Such high level of antioxidant activity could be attributed to high content of the measured bioactive compounds such antioxidant vitamins and bioactive compounds. In similar study, by Makaepea, *et al.* [54] found that watermelon contains phytochemicals such as lycopene, vitamin C, β-carotene, and total polyphenolic content that possess anti-inflammatory, anticancer, and antioxidant properties. Phytochemicals such as lycopene and β-carotene have shown to have antioxidant, anti-inflammatory, and hypotensive properties; therefore, their inclusion on diet results in positive effects on the human body [55, 56]**.** For polysaccharides, several biological activities including anticoagulant, antithrombotic, anti- inflammatory, anti-obese, antiviral, immune system-boosting properties and anti-osteoporosis, anticarcinogenic, hypocholesterolemic and hypolipidemic responses [57]**.** Other bioactive compounds i.e. Phenolics, flavonoids, carotenoids, anthocyanin's and tannins which were determined in WME have proven that posses different biological activities (antioxidant, anticancer and anti-inflammatory activities) wherefore they have owned important roles in preventing and/or treating many diseases such as diabetes, atherosclerosis, cancer, obesity, bone, anemia and aging [32, 57-60]. Regarding the chlorophylls, WME have remarkable amount which provides nutritional benefits to the body and helps keep you healthy including healthy bones, strong muscles, maintaining normal blood pressure and needs for the blood to clot properly [50]**.** Finally, WME samples were recorded antioxidant vitamins (A, C and E). Vitamin A reported a likely antioxidant role in an indirect way and could play a role in protecting the body against OS damage [61]. Vitamin C is a powerful antioxidant with the ability to mop up free radicals within, and outside the cell which is by acting directly on peroxyl radicals or indirectly by boosting the antioxidant properties of vitamin E [62]**.** From the above it is clear that WME contains a lot of bioactive compounds in addition to many vitamins, which is characterized by having many important biological effects. All of these factors suggest the use of this extract for many studies related to cancer, such as what was accomplished in the next part of this study "studying the effect of WPEE on the molecular damage induced by B[*a*]P in liver cells".

3.3. Liver Cells Viability

Table (3) and Fig. (2) illustrated effect of simultaneously treated with B[a]P and WPEE on liver cell viability. Significantly increase in mean percentage number of dead cells in liver cells treated with B[*a*]P (84.5±2.2) as compared with normal liver cells (4.3±2.2), while liver cells simultaneously treated with B[a]P and WPEE which concentrations (25, 75, 75 and 100 µg/ml) showed significant decrease in mean percentage of dead cells with values 69±1.7, 54.5±2.5, 40±2.7 and 80±1.7, respectively as compared with liver cells treated with B[a]P. The toxicity of B[a]P towards organisms has been widely reported using liver cells as an in vitro model system [2, 59]**.** Confirmed that a concentration of 50 µM B[a]P was observed to strongly inhibit the growth of 52.78% hepatic epithelial cells. All of those studies indicated that the reason behind the increasing of dead cells which treated with B[a]P compared with normal cells is that it showed great cytotoxicity effects (Cell wall membrane integrity and lysosomes and mitochondria dysfunction) that causes the death and destruction of a large percentage of cells when present at a specific dose. Additionally, oxidative stress has been regarded to be responsible for the cytotoxicity following B[a]P exposure, as B[a]P causes cellular oxidative damage by increasing the levels of free radicals in the cells [59, 63]**.** In contrast to cells exposed to WPEE, we notice a noticeable decrease in the number of dead cells and an increase in the number of live cells. This is probably due to the protective ability of WPEE because it contains many antioxidants such as phytochemicals (phenolics, polysaccharides, carotenoids) and vitamins A, E and C) that work to protect cells from oxidative damage and cytotoxicity effects. Such evidence was confirmed by our previous data [59, 64]**.** Also, throughout cytotoxic challenge of B[a]P, a fall in glutathione (GSH) and albumin (Alb) content in liver cells were noted, [59]**.** GSH is an important biological antioxidant and albumin production is often employed as a marker of hepatocyte metabolic activity *in vitro* [65]**.** Such cytotoxic effects observed by B[*a*]P treatment accompanied by a concomitant increased in the liver cells lipid peroxidation (MDA content) [59]**.** The same study confirmed that all of the cultured liver cells functions were improved as the co-treatment with B[a]P and plant parts extracts (prickly pear peel, turmeric rhizomes, red onion skin and eggplant peel) methanolic extracts, which contains most of the bioactive compounds and vitamins contained in the WPEE, in a dose-dependent manner and cells grown in media supplemented with those extracts appeared more tolerant to the cytotoxin, i.e. B[*a*]P.

3.4. Electrophoretic Pattern of Nucleic Acids (RNA and DNA)

3.4.1. Ribonucleic Acid (RNA)

Result indicated an increase in mean value of maximal optical density of RNA in liver cells treated with DEMSO (149.3±3.7) compared to normal liver cells (111± 2.5). While, liver cells treated with B[*a*]P showed significantly decrease ($P \leq 0.05$) in mean number of maximal optical density with value 81.5±3.6 compared to normal cells. On the other hand, mean of maximal optical density in liver cells simultaneously treated with B[*a*]P (10 µM) and WPEE (25, 50, 75 and 100 µg/ml) indicated significantly increase with values92.7±1.8, 89.7±4, 94±3.9 and 84±3.9, respectively compared to liver cells treated with B[*a*]P (100µg) only (Table 4, Fig. 3. and Photo 1)

3.4.2. Deoxyribonucleic Acid (DNA)

As shown in Table (5), Fig. (4). and Photo (2), result recorded a decrease in mean of maximal optical density of intact DNA in liver cells treated with B[*a*]P compared with normal liver cells (148±2.5). Also, liver cells simultaneously treated with B[*a*]P (100µg) and WPEE (25, 50, 75 and 100 µg/ml) showed a significant decrease i mean of maximal optical density of intact DNA with values 108.7±2.9, 94.3±3.7, 118.7±2.9 and 83.5±3.6, respectively as compared with normal liver cells. On the other hand, mean of maximal optical density of fragmented DNA illustrated a significant decrease in liver cells treated with B[*a*]P (61±2.5) as compared with normal liver cells (108.7±5.7). But, liver cells simultaneously treated with B[*a*]P (10µM) and WPEE (100 µg/ml) showed an increase in mean of maximal optical density of fragmented DNA with value 66±2.9 as compared with liver cells treated with B[*a*]P. While, mean of maximal optical density of fragmented DNA in liver cells simultaneously treated with B[*a*]P (100µg) and WPEE (25, 50 and 75 µg/ml) indicated a significant increase as compared with liver cells treated with B[*a*]P.

Carcinogenesis/ mutagenesis of B[*a*]P is associated with oxidative stress, inflammation, angiogenesis, and metastasis [2, 59, 63, 66, 67]. The oxidative stress is from increased reactive oxygen species (ROS) and reduced counteracting antioxidants, The ROS attack the biomolecules such as DNA, RNA, proteins and lipids directly. The oxidative damage to DNA and RNA includes the formation of 8-oxo-7, 8-dihydro-20-deoxyguanosine (8-oxo-dG) or 8-oxo-7, 8-dihydro-20-guanosine (8-oxo-G), which is increased in cancers [2]**.** Studies related to this subject indicated that B[*a*]P treatment triggers a marked increase in CYP1 expression and activity generating, upon metabolic activation, DNA adducts, DNA damage and double-strand breaks (DSBs) in human T lymphocytes [68]**.** Such factors induce gene mutations which could consequently drive a cancer process. Another studies published earlier have implicated that BaP metabolites (phase I) in the causation of DNA damage. It has been shown that diol epoxides of BaP form stable adduct in *in vitro*, [2] and *in vivo* [69] systems. Benzo(*a*)pyrene metabolites such as B[*a*]P 7,8-dione have been reported to cause oxidative DNA damage**,** [70]**.** Other quinones metabolites may also be responsible for the formation of lesions, as we have seen greater concentrations of B[*a*]P 3,6-dione and B[*a*]P 6,12-dione at 10- and 25-µM concentrations, compared to lower concentrations of B[a]P. Consistent with the expression of phase I enzymes, elevated concentrations of B[a]P metabolites were generated, contributing to the formation of DNA lesions and stable DNA adducts, which were also B[*a*]P concentration-dependent [71]**.**

In the present study, WPEE exhibited anticarcinogenic effect against B[a]P in liver cells through prevent damage to macromolecules, such as_DNA and RNA measured by Electrophoretic pattern. Such effect could be

attributed to the high levels content of different bioactive compounds including phytochemicals (phenolics, flavonoids, carotenoids, lycopene , tannins, anthocyanin's, polysaccharides and chlorophyll) and vitamins (A, C and E) found in such extract. Our previous and present data indicated that all of those compounds exert different highly biological activities including antioxidant, scavenging free radicals and inhibiting the lipid peroxidation [23, 30, 72] Due to their biological properties, WPEE can inhibit the cellular oxidative stress and is prevent the oxidative damage to DNA/RNA.

Studies published earlier have implicated that foods contained different bioactive compounds such as found in WPEE have potential impact(s) on the preventing of the damage of DNA/RNA and other macromolecules. A study by Rangel-Huerta, *et al.* [73] found that the use of orange juice polyphenols in obese subjects led to a decrease in urinary 8-OH-G as one of the markers of DNA damage. Also, Spadafranca, *et al.* [74] evaluated the property of spinach powder as a source of polyphenols in the diet of hyperlipidemic rats to evaluate its effect on preventing the rate of failure in the DNA sequence. In addition, the results of the study by Spadafranca, *et al.* [74] indicated that the consumption of dark chocolate polyphenols reduced the damage to DNA from mononuclear cells in healthy people. Other bioactive compounds such as carotenoids, the results of various studies indicate that increased levels of that compounds especially β- carotene, can reduce the amount of damage to DNA [75, 76]. For vitamins, vitamin C reduce the chromosomal abnormalities, which can reduce DNA defects and decrease the amount of 8-oxodG, as one of the indicators of DNA damage [77]**.** According to studies on the antioxidant properties of vitamin E, the role of this vitamin in preventing DNA damage is significant [78, 79]**.** Interactive effects, taking β-carotene together with vitamins E and C, has a protective effect against damage to DNA which was produced by gamma rays [80]**.**

4. Conclusion

B[*a*]P is associated with oxidative stress which means increased ROS and reduced counteracting antioxidants. In liver cells, the ROS attack the biomolecules such as DNA, RNA, proteins and lipids directly which could consequently drive a cancer process. Antioxidant compounds include polyphenols, polysaccharides, carotenoids, and some vitamins such as vitamins A, C and C were determined in WPEE. These compounds can prevent damage to macromolecules, such as DNA and RNA through several mechanisms including neutralize/scavenge free radicals. Also, suggesting a positive relationship between WPEE concentrations applied and DNA damage prevented was recorded. These findings provide a basis for the use of WPEE for the prevention and/or treatment of xenobiotics inducing DNA and RNA damage. Also, we recommended the using of watermelon skin in different forms, powder and extracts, in our daily live dishes, beverages and pharmacological formulae.

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Conflict of Interests

Authors declared no competing of interest whatsoever.

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Table-1. Proximate chemical composition and total energy value of watermelon peel powder

Component (g/100g)	Content
Moisture	9.86 ± 0.82
Total protein	7.12 ± 1.54
Crude fat	0.32 ± 0.12
Ash	3.04 ± 0.39
Crude Fiber	2.77 ± 0.73
Carbohydrate	76.89 ± 2.69
Energy (Kcal/100g)	338.92 ± 9.56

*Each value represents the mean of three replicates ±SD.

Table-2. Total content of bioactive compounds, vitamins and antioxidant activity in WPEE

Component	Mean ±SD
bioactive compounds:	
Phenolics (mg GAE/g dry extract)	4.01 ± 0.56
Flavonoids (mg CE/ g dry extract)	1.12 ± 0.17
Carotenoids (mg BCE/g dry extract)	1.26 ± 0.29
Lycopene (µg/ g dry extract)	80.51 ± 5.73
Tannins (mg CE/g dry extract)	0.92 ± 0.13
Anthocyanin's (mg, CCy3G E/100g dry extract).	0.52± 0.09
Polysaccharides (mg SE/ g dry extract)	0.86 ± 0.21
Chlorophyll (µg/g dry extract)	85.20 ± 4.69
Vitamins:	
Vitamin A (Retinol, mg/g dry extract)	2.76 ± 0.89
Vitamin C (Ascorbic acid, mg/g dry extract)	0.89 ± 0.12
Vitamin E (α-tocopherol, mg/g dry extract)	0.37 ± 0.08
Antioxidant activity (AA):	
AA [% of a-tocopherol (50mg/ml)]	74.50 ± 3.78
AA [% of BHT (50mg/ml)]	83.61 ± 2.55

*Each value represents the mean of three replicates ±SD. GAE, gallic acid equivalent, CE, catechin equivalent, BCE, β-carotene equivalent, CCy3GE, Cyanidin 3-glucoside equivalent. and SE, starch equivalent.

Table-3. Effect of simultaneously treated with B[*a*]P and WPEE on liver cell viability

Groups	Live cells (%)	Dead cells (%)
Normal liver cells	95.7± 2.6	4.3±2.2
Liver cells + DEMSO	39±3.3*	61±2.7*
Liver cells + B[*a*]P (10 µM)	15.5±1.9*	84.5±2.2*
Liver cells + B[*a*]P (10 µM) + WPEE (25 µg/ml)	31±2.7*$	69±1.7*$
Liver cells + B[*a*]P (10 µM) + WPEE (50 µglml)	45.5±3.4*$	54.5±2.5*$
Liver cells + B[*a*]P (10 µM) + WPEE (75 µg/ml)	60±3.5*$	40±2.7*$
Liver cells + B[*a*]P (10 µM) + WPEE (100 µg/ml)	22.3±4.3*$	80.7±1.7*$

Data are expressed as Mean ± SD. * Significant difference compared to normal liver cells at ($P \leq 0.05$). $ Significant difference compared to liver cells treated with B[*a*]P (100µg) at ($P \leq 0.05$).

Table-4. The mean of maximal optical density (MOD) of apoptotic fragments of DNA in liver cells culture simultaneously treated with benzo(*a*)pyrene and water melon peel extract

Groups	MOD of Intact DNA	MOD of fragmented DNA
Normal liver cells	148± 2.5	108.7±5.7
Liver cells + DEMSO	161±2.5*	113.5±3.1*
Liver cells + B[*a*]P (10 µM)	73.7±2.9*	61±2.5*
Liver cells + B[*a*]P (10 µM) + WPEE (25 µg/ml)	108.7±2.9*$	85±4*$
Liver cells + B[*a*]P (10 µM) + WPEE (50 µg/ml)	94.3±3.7*$	73±2.5*$
Liver cells + B[*a*]P (10 µM) + WPEE (75 µg/ml)	118.7±2.9*$	118±2.5*$
Liver cells + B[*a*]P (10 µM) + WPEE (100 µg/ml)	83.5±3.6*$	66±2.9*

Data are expressed as Mean ± SD. * Significant difference compared to normal liver cells at ($P \leq 0.05$). $ Significant difference compared to liver cells treated with B[*a*]P (10 µM) at ($P \leq 0.05$).

Table-5. The mean of maximal optical density (MOD) of total RNA in liver cells culture simultaneously treated with benzo(*a*)pyrene and water melon peel extract

Groups	MOD of RNA
Normal liver cells	111± 2.5
Liver cells + DEMSO	149.3±3.7*
Liver cells + B[*a*]P (10 µM)	81.5±3.6*
Liver cells + B[*a*]P (10 µM) + WPEE (25 µg/ml)	92.7±1.8*$
Liver cells + B[*a*]P (10 µM) + WPEE (50 µg/ml)	89.7±4*$
Liver cells + B[*a*]P (10 µM) + WPEE (75 µg/ml)	94±3.9*$
Liver cells + B[*a*]P (10 µM) + WPEE (100 µg/ml)	84±3.9*

Data are expressed as Mean ± SD. * Significant difference compared to normal liver cells at ($P \leq 0.05$). $ Significant difference compared to liver cells treated with B[*a*]P (10 µM) at ($P \leq 0.05$).

Fig-1. Trans-section of watermelon (*Citrullus lanatus*) fruit showing the various parts including, skin, rind, peel, pulp and seeds

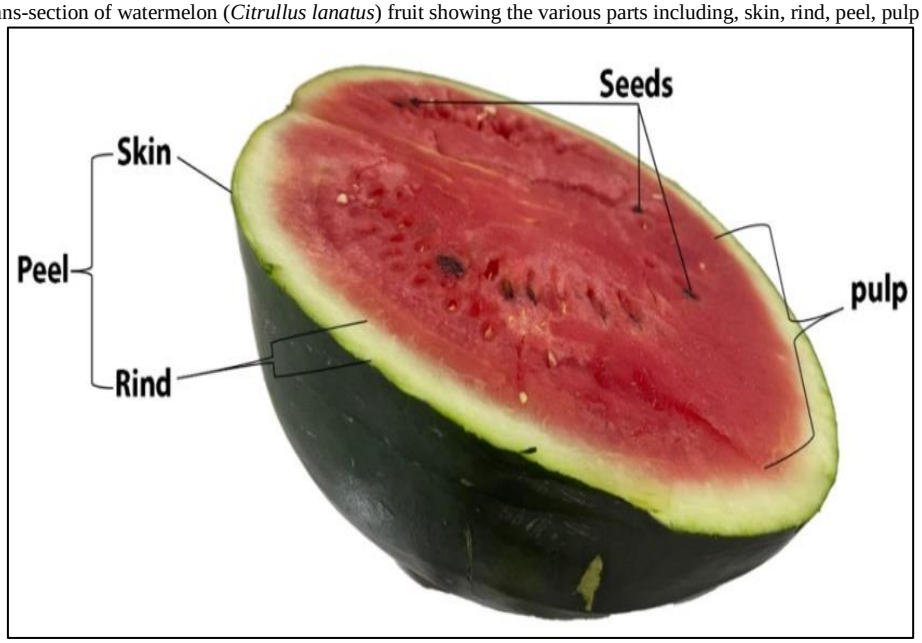


Fig-2. Effect of simultaneously treated with Benzo[*a*]pyrene and water melon peel extract on liver cell viability. LC, liver cells, DEMSO, dimethysulfoxide, WPEE, watermelon peel ethanolic extract

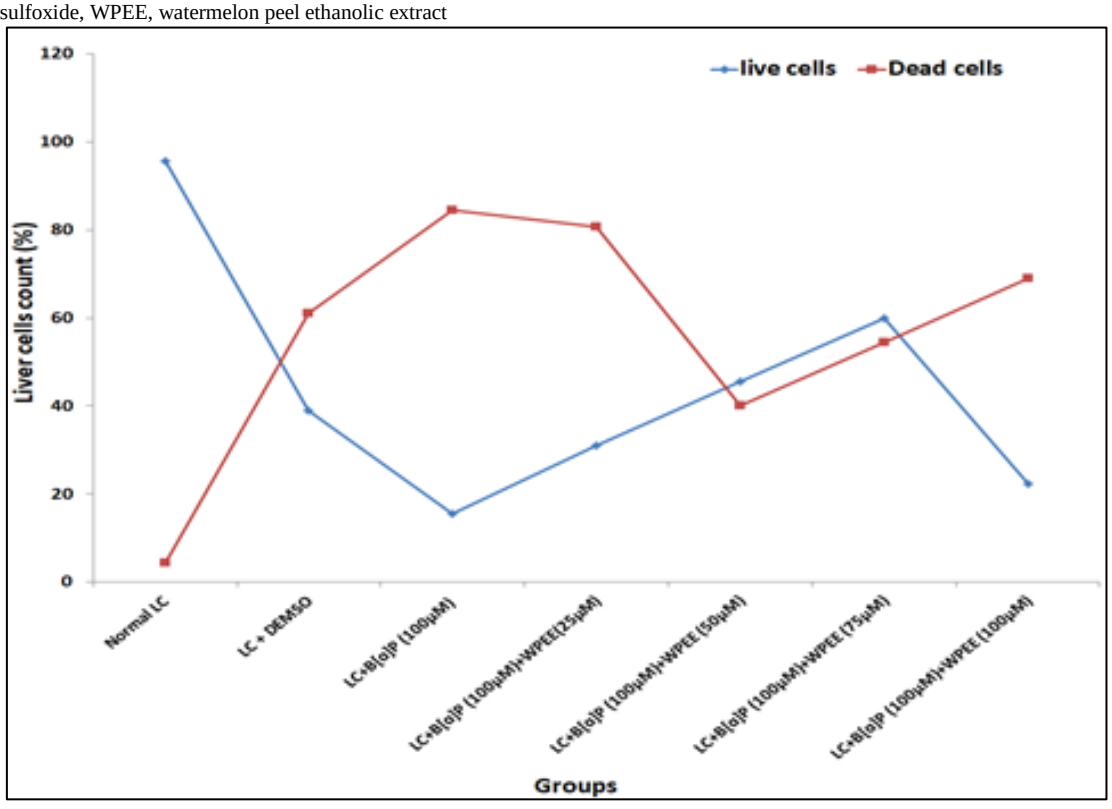


Fig-3. Electophoretic pattern of nucleic acid in liver cells culture simultaneously treated with benzo(*a*)pyrene and water melon peel extract

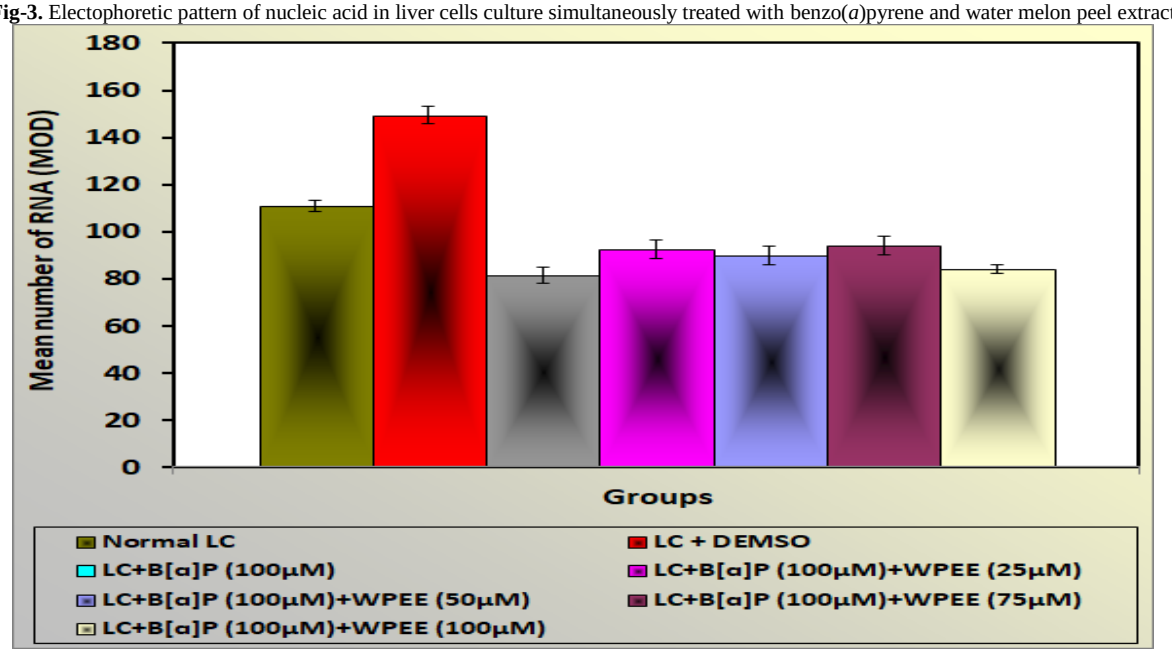


Fig-4. DNA fragmentation in liver cell culture simultaneously treated with benzo(*a*)pyrene and water melon peel extract

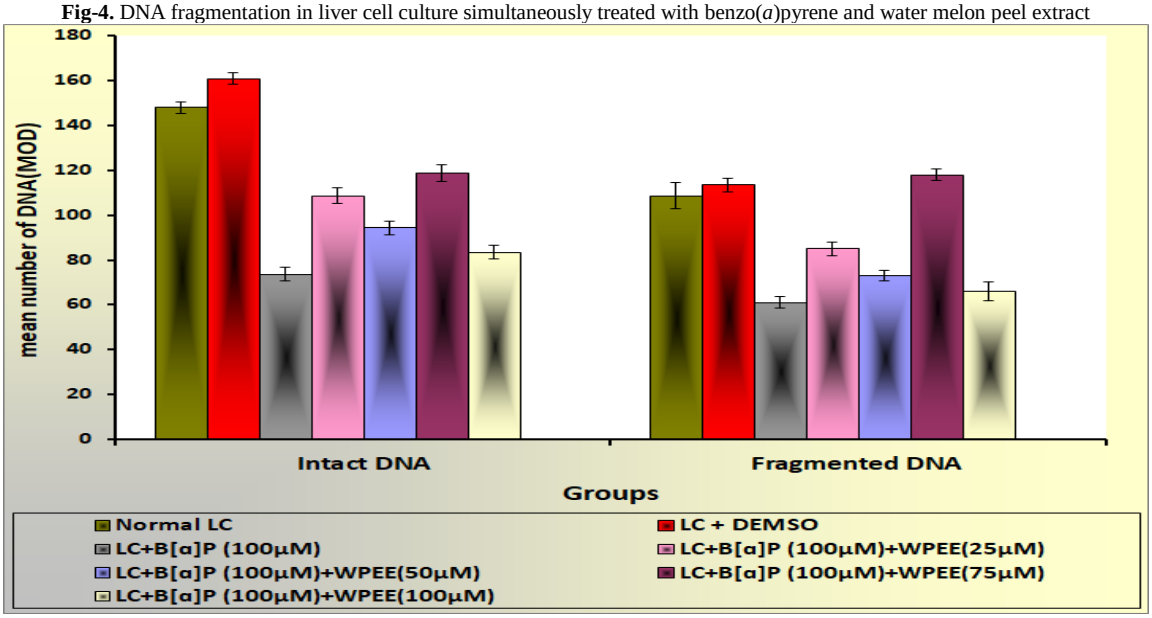
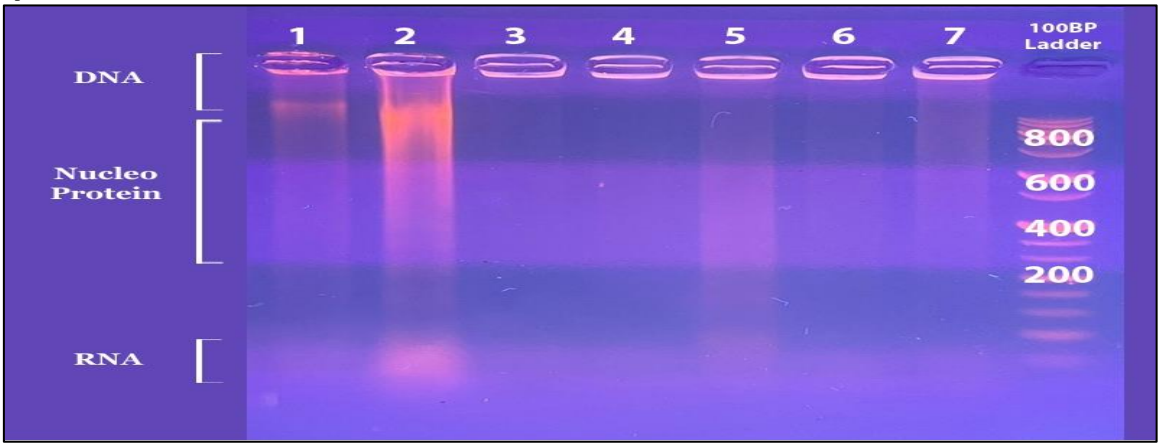
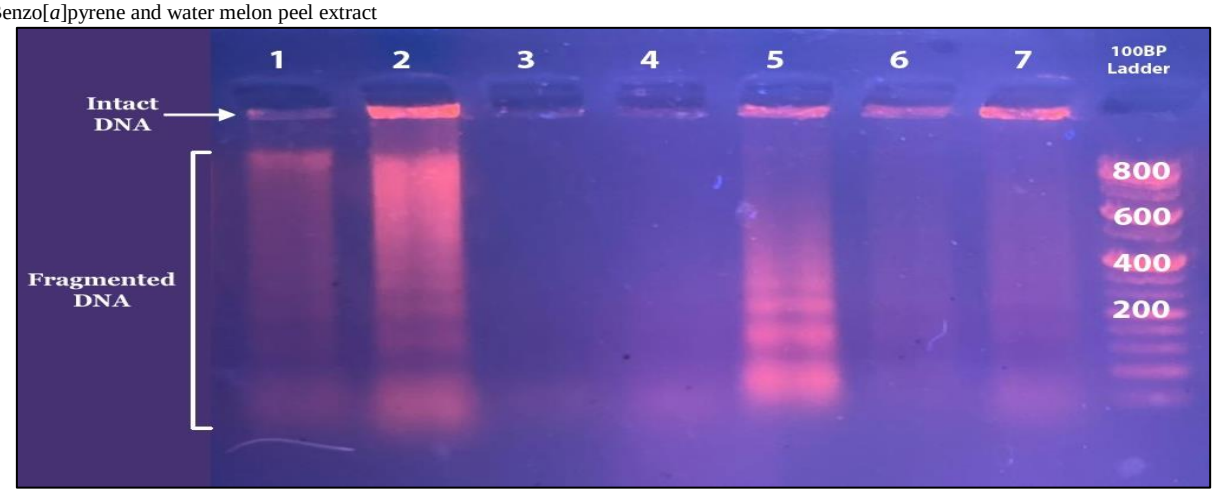


Photo-1. The mean of maximal optical density (MOD) of total RNA in liver cells culture simultaneously treated with benzo(*a*)pyrene and water melon peel extract



Lane 1: Normal liver cells; Lane 2: Liver cells treated with DEMSO; Lane 3: Liver cells treated with B[*a*]P (10 µM); Lane 4: Liver cells treated with B[*a*]P plus WPEE (100 µg/ml); Lane 5: Liver cells treated with B[*a*]P (10 µM) plus WPEE (75 µg/ml); Lane 6: Liver cells treated with B[*a*]P (10 µM) plus WPEE (50 µg/ml); Lane 7: Liver cells treated with B[*a*]P (10 µM) plus WPEE (25 µg/ml).

Photo-2. The mean of maximal optical density (MOD) of apoptotic fragments of DNA in liver cells culture simultaneously treated with Benzo[*a*]pyrene and water melon peel extract



Lane 1: Normal liver cells; Lane 2: Liver cells treated with DEMSO; Lane 3: Liver cells treated with B[*a*]P (10 µM); Lane 4: Liver cells treated with B[*a*]P plus WPEE (100 µg/ml); Lane 5: Liver cells treated with B[*a*]P (10 µM) plus WPEE (75 µg/ml); Lane 6: Liver cells treated with B[*a*]P (10 µM) plus WPEE (50 µg/ml); Lane 7: Liver cells treated with B[*a*]P (10 µM) plus WPEE (25 µg/ml).